Summary of 2013 wild rice mesocosm experiment workplan for John Pastor
May 31, 2013

Task: Outdoor mesocosm experiments to determine responses of wild rice growth to a range of sulfate concentrations.

We will make use of an ongoing experiment on wild rice response to sulfate additions begun in the spring of 2011 performed at the University of Minnesota Duluth Research and Field Studies Station, Duluth, MN in conjunction with the Fond du Lac Band of Lake Superior Chippewa and the Grand Portage Band of Chippewa. A second year of study using these same mesocosms was completed in 2012. The same experimental design will be conducted in 2013 under sponsorship of the MPCA.

The following describes how the experiment was set up in 2011. Mesocosms were prepared using polyethylene stock tanks (Rubbermaid #4242, 378 L capacity, 132 cm long x 79 cm wide x 63 cm deep). The tanks were partly filled with 10 cm of clean washed sand and covered with approximately 10 cm of surface sediment collected from natural wild rice beds from a lake on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota. The collected sediment was homogenized in a large stock tank prior to distribution in the mesocosm tanks. Each mesocosm tank was buried to ground level and connected by a drain pipe to a 20 L polyethylene overflow bucket. Water levels in the tanks are controlled using a drain pipe set at 23 cm above the sediment surface. Test sulfate concentrations were selected at 0, 50, 100, 150, and 300 mg $SO_4^{-2} \cdot L^{-1}$. The experimental design uses a total of 30 tanks divided into six replicate tanks per treatment with 5 treatment levels (Control plus 4 sulfate concentrations). Each mesocosm tank was assigned a designated treatment and replicate, and was positioned using a randomized block design. Low standard deviations of analyses of three volumetric samples of the mixed sediment indicated a homogenous material (% C = 12.18 ± s.d. 1.00, % N = 1.07 ± s.d. 0.02; Walker et al. 2010). Sediment bulk density was 0.27 $g \cdot cm^{-3}$ ± s.d. 0.01 (Walker et al. 2010). These values are comparable to those of other wild rice beds (Keenan and Lee 1988, Day and Lee 1989).

During the growing season, water levels are maintained weekly through additions of water up to the height of the drain pipe or by allowing water to drain through the drain pipe set at 23 cm above the sediment surface. Water used to fill and supplement tank levels is obtained from a nearby wellpoint. Rainfall N concentrations as NO_3-N and NH_4-N ranged from 0.2 – 1.99 $\mu g \cdot mL^{-1}$ while the NO_3-N and NH_4-N concentrations in the well water are always < 0.2 $\mu g \cdot mL^{-1}$ (Walker et al. 2010). Sulfate concentrations in well water are between 0 and 10 $\mu g \cdot mL^{-1}$. The sediments comprise an inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter will remain submerged year round with water levels set at approximately 20 cm in late fall.

The initial test with the mesocosms was planted with wild rice in mid to late spring 2011 from seeds obtained from the Grand Portage Reservation in northeastern Minnesota.

Seedlings in each tank are thinned (removed from the tank) to a density of 30 plants per tank during the spring when the plants have reached the floating leaf stage. At this time, a large number of seedlings are observed growing and these plants are thinned to this desired density. Plants are thinned to achieve relatively uniform spacing within each tank. An additional thinning criterion is to remove all plants within 5 cm of the outside wall of the tank. Additional plants that emerge as floating leaf are thinned as needed over about a two week period. A total count of seedlings removed from each tank is recorded.

Sulfate concentrations are monitored on a weekly basis and adjusted as needed. Sulfate is added to the tanks as solutions of sodium sulfate (Na_2SO_4) in the appropriate concentrations as reported above (Fisher Chemical S421). To do this, the required amount of sodium sulfate is weighed and dissolved in 1 to 2 liters of water from the filled tank. The stock solution is then diluted and added back to the tank with gentle mixing. Subsequent sulfate measurement and adjustments to the sulfate concentration are made weekly if needed.

During the growing season water is sampled from each tank every week, the overflow buckets, and rain gauge, and sulfate concentrations are analyzed using a Lachat Autoanalyzer by a barium chloride turbidimetric method (Lachat #10-116-10-1-A, USEPA/NPDES equivalent method). Water samples are also analyzed for sulfate, sulfide, total nitrogen and phosphorous, ammonium, and nitrate. In addition, tank water pH and temperature is measured in each tank every 2 weeks using a portable meter.

Five plants in each tank are chosen in early summer for detailed measurements throughout the growing season and sampling at the end of the growing season. These plants are selected by first dividing the tank into five equal areas and professional judgment is used to select an average-sized plant growing within each section. Heights of these plants are measured every two weeks throughout the growing season. In late August to September, seeds are collected by "milking" the seed head every two to three days during the period of seed dispersal. At the end of the growing season these five plants are collected by gently excavating the root system from the sediment. The seeds from the five sampled plants are dried at 60^{o} C, counted and weighed. In addition, all aboveground plant material is harvested from each tank by cutting the stems at the sediment surface. All stems are counted in these samples to determine end of growing season plant density. In addition, total weight of the biomass of plants is measured, and a representative subsample (approximately 10% of mass), employing professional judgment, is removed to determine wet:dry ratios for moisture correction—a ratio that has been relatively constant from tank to tank over the past two years. Root:shoot ratios and seed weights and numbers from the five sampled plants are applied to total aboveground plant weights and total plant numbers to determine total root and seed production in each tank. All aboveground plant material, except for the five sample plants, which are retained for possible analysis, is returned to each tank. Only 10% of the aboveground plant material is dried because it has been observed that dried wild rice plants degrade in the tank at a slightly higher rate than occurs without drying. Total end of season biomass and relative growth rate are calculated as endpoints.

All data will be entered for statistical analysis in Excel spreadsheets. The effect of sulfate levels on wild rice seed, shoot, and root growth is examined using a randomized complete block analysis of variance. Photographs are taken periodically during the growing season to visually document growth and condition of the stems, roots, and seeds and overall experimental design.

Additional Measurements Associated with the 2013 Mesocosm Experiments

Additional sampling of bulk sediment, sediment pore water and overlying water will be performed using the methods described for the field studies being conducted by Drs. Amy Myrbo and Nate Johnson.

References

Day WR, Lee PF. 1990. Ecological relationships of wild rice, *Zizania aquatica.* 9. Production in organic-flocculent sediments.Canadian Journal of Botany 68: 1542-1548.

Harmon ME, Lajtha K (1999) Analysis of detritus and organic horizons for mineral and organic constituents. *in* Robertson GP, Coleman DC, Bledsoe CS, Sollins P Standard soil methods for long-term ecological research. Oxford University Press, New York, pp 148-150.

Keenan TJ, Lee PF. 1988. Ecological relationships of wild rice, *Zizania aquatica.* 7. Sediment nutrient depletion following introduction of wild rice to a shallow boreal lake. Canadian Journal of Botany 66: 236-241.

Li, S., I.A. Mendelssohn, H. Chen, and W.H. Orem. 2009. Does sulphate enrichment promote the expansion of *Typha domingensis* (cattail) in the Florida Everglades? Freshwater Biology 54: 1909-1923.

Vogt, D. J. 2010. Wild Rice Monitoring and Abundance in the 1854 Ceded Territory (1998-2010). 1854 Treaty Authority, Duluth, MN.

Walker, R. D., J. Pastor, and B. Dewey. 2010. Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.*)* in northern Minnesota. Ecosystems 13: 485-498.